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Regional Differences in Bioelectric Properties and Ion Flow in Excised Canine Airways

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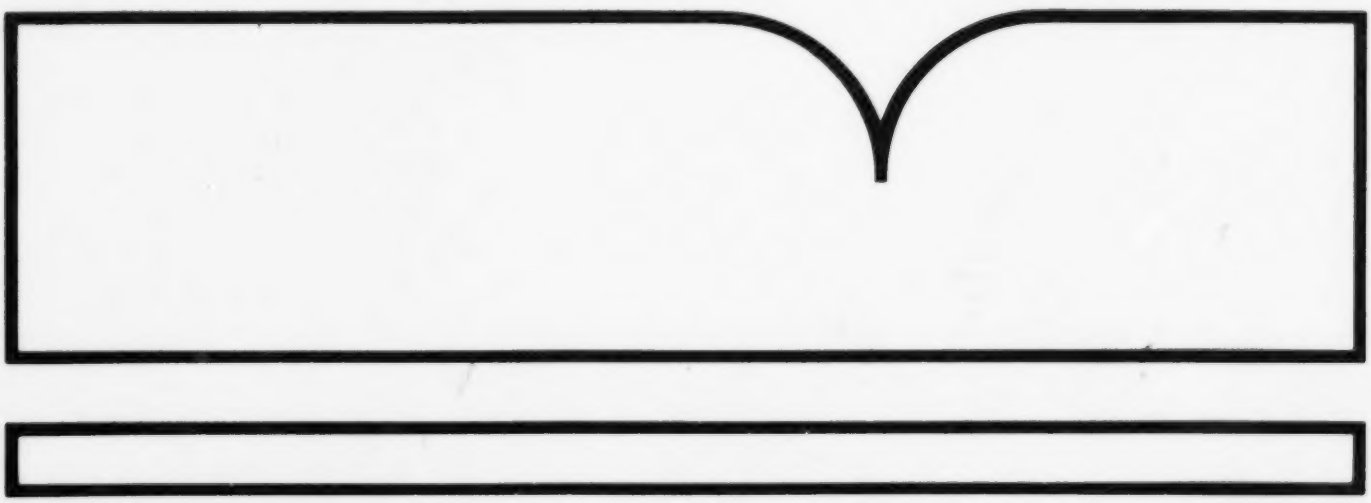

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16. ABSTRACT

Absorption of airway liquid, as it moves from the large surface area of distal airways to more central airway surfaces, is considered to be essential for regulation of the depth of airway liquid. The short-circuited excised canine trachea, however, secretes Cl^- and absorbs a lesser amount of Na^+. We compared bioelectric properties and unidirectional ion flows across epithelia excised from trachea and mainstem and 4th- to 6th-generation bronchi. Compared with trachea, 4th- to 6th-generation bronchi exhibited a lower potential difference (8.9 compared with 35 mV) and higher direct-current conductance (6.9 compared with 2.1 $mS \cdot cm^{-2}$). Corresponding values for main-stem bronchus were intermediate. Under short-circuit conditions, the trachea secreted Cl^- (1.75 $\mu eq \cdot cm^{-2} \cdot h^{-1}$) and absorbed Na^+ (1.15 $\mu eq \cdot cm^{-2} \cdot h^{-1}$). K^+ fluxes were symmetric. Main-stem bronchus exhibited a similar pattern of ion flow. Subsegmental bronchi absorbed 2.0μeq $Na^+ \cdot cm^{-2} \cdot h^{-1}$ and secreted 0.04 μeq $K^+ \cdot cm^{-2} \cdot h^{-1}$, but net Cl^- transport was absent. Under open circuit, Na^+ absorption persisted in all regions, but net Cl^- secretion dissappeared in the trachea and main-stem bronchi. Na^+ absorption was uniformly abolished by addition of ouabain (3×10^{-5} M) to the submucosal bath. We conclude that NaCl absorption dominates resting ion translocation in canine bronchi and that liquid absorption probably occurs at these sites in vivo.

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Regional differences in bioelectric properties and ion flow in excised canine airways

R. C. BOUCHER, M. J. STUTTS, AND J. T. GATZY
Departments of Medicine and Pharmacology, University of North Carolina, Chapel Hill, North Carolina 27514

BOUCHER, R. C., M. J. STUTTS, AND J. T. GATZY. *Regional differences in bioelectric properties and ion flow in excised canine airways.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 51(3): 706–714, 1981.—Absorption of airway liquid, as it moves from the large surface area of distal airways to more central airway surfaces, is considered to be essential for regulation of the depth of airway liquid. The short-circuited excised canine trachea, however, secretes Cl^- and absorbs a lesser amount of Na^+. We compared bioelectric properties and unidirectional ion flows across epithelia excised from trachea and mainstem and 4th- to 6th-generation bronchi. Compared with trachea, 4th- to 6th-generation bronchi exhibited a lower potential difference (8.9 compared with 35 mV) and higher direct-current conductance (6.9 compared with 2.1 $mS \cdot cm^{-2}$). Corresponding values for main-stem bronchus were intermediate. Under short-circuit conditions, the trachea secreted Cl^- (1.75 $\mu eq \cdot cm^{-2} \cdot h^{-1}$) and absorbed Na^+ (1.15 $\mu eq \cdot cm^{-2} \cdot h^{-1}$). K^+ fluxes were symmetric. Main-stem bronchus exhibited a similar pattern of ion flow. Subsegmental bronchi absorbed 2.0 $\mu eq\ Na^+ \cdot cm^{-2} \cdot h^{-1}$ and secreted 0.04 $\mu eq\ K^+ \cdot cm^{-2} \cdot h^{-1}$, but net Cl^- transport was absent. Under open circuit, Na^+ absorption persisted in all regions, but net Cl^- secretion disappeared in the trachea and main-stem bronchi. Na^+ absorption was uniformly abolished by addition of ouabain (3×10^{-5} M) to the submucosal bath. We concludé that NaCl absorption dominates resting ion translocation in canine bronchi and that liquid absorption probably occurs at these sites in vivo.

ion transport; bronchi; airway secretions

THE FUNCTIONAL ROLE of the pulmonary epithelium in the regulation of the volume and composition of lung surface liquid is not well understood. In the fluid-filled fetal lung of mammals, liquid secretion into the lumen is driven by the active transport of Cl^- (24, 29), an ion translocation that has also been described for the alveolar epithelium of the bullfrog lung (13). Ion secretion has not been identified in the alveolus of adult mammalian lung, even though surfactant is secreted by type II pneumocytes (18) and maximal surfactant activity at the air-epithelial surface probably requires the presence of an electrolyte solution (27).

Although it is thought that liquid is secreted by distal epithelial surfaces, small airways, and/or alveoli (20), liquid absorption has been postulated by Kilburn (3, 19) to occur in proximal airways. This postulate is based on *1*) histological evidence for a surface-liquid layer of uniform depth, *2*) relatively uniform (within 10-fold) regional velocities of markers of surface liquid movement toward the mouth, and *3*) the large surface area of distal airway surfaces compared with the smaller area of central airways. Hence, failure to remove a portion of the surface liquid that moves toward central airways would theoretically lead to airway occlusion at the point of minimal cross sectional diameter, generally second- to third-generation bronchi.

Attempts to directly measure electrolyte and water flow across airway epithelia, however, have been limited to the trachea. The posterior epithelial membrane of the canine trachea has been used in many of these studies. When this tissue is mounted in an Ussing chamber and the spontaneous transepithelial electric potential difference (PD) of about 30 mV (lumen negative) is reduced to zero (i.e., short-circuited), the trachea secretes Cl^- and absorbs Na^+ (23). Net ion flows were not detected across the unstimulated trachea under open-circuit conditions, that is, an electrical gradient comparable to that measured in vivo (4, 23). However, Cl^- and water secretion by the excised canine trachea can be induced under open-circuit conditions by agents of several pharmacologic classes (22, 30). Some of these agents or laryngeal nerve stimulation induce secretion in vivo (8, 9). Accordingly, net electrolyte secretion by the canine trachea in the presence of a spontaneous transepithelial PD may not be a basal property and appears to require the participation of regulatory factors.

Several direct measurements suggest that the pattern of epithelial ion transport and/or permeability may be different in more distal airways. Compared with the relatively high PD that has been measured across the trachea of several species in vivo and in vitro, the spontaneous transepithelial voltage of lobar and subsegmental bronchi is substantially lower (4). In addition, analysis of fluid from the surface of canine trachea and bronchi have revealed differences in water and electrolyte composition. Whereas tracheal surface liquid is characterized by a higher NaCl concentration and osmolality, bronchial Na^+ and Cl^- concentrations are lower and K^+ and HCO_3^- concentrations are higher than the corresponding values for plasma (16). Our study was designed to evaluate ion flows across canine bronchi in vitro and to compare these results with those obtained from the trachea under identical conditions.

METHODS

Animals and tissue preparation. Male mongrel dogs (20–28 kg) were anesthetized with intravenous pentobarbital sodium (25 mg/kg), and an incision was made that exposed the trachea and sternum. The animal was killed with an overdose of pentobarbital, the thorax was opened, and the trachea with attached lungs was excised and placed in cold Ringer solution. Transverse sections, 4 cm in length, were cut from the trachea, and the posterior portion was stripped of muscle and subepithelial tissue with fine scissors. The right and left main-stem bronchi were isolated and the subcartilaginous tissue dissected free. Lobar bronchi were identified, and attendant intraparenchymal bronchi were uncovered by sharp dissection. Two segmental and/or subsegmental bronchi, fourth to sixth generation, 7–8 mm in diameter, were isolated from each lung (16, 33). Specimens were taken randomly from seven lobes.

Tissue mounting, bathing solutions, and bioelectric measurements. All airway specimens (trachea, mainstem bronchi, and 4th- to 6th-generation bronchi) were mounted in Ussing chambers with an aperture of 0.53 cm^2. The specimens were bathed on both sides by 8 ml of mammalian Krebs-bicarbonate Ringer (in mM: Na^+ 140, Cl^- 120, K^+ 5.2, HCO_3^- 25, HPO_4^{2-} 2.4, $H_2PO_4^-$ 0.4, Ca^{2+} 1.1, Mg^{2+} 1.2, glucose 10) that was warmed to 38°C, gassed with 95% O_2-5% CO_2 to maintain solution pH at 7.4, and circulated by gas-lift pumps. In a few experiments, $NaHCO_3$ and Na phosphate were replaced by NaCl. This unbuffered solution was bubbled with 100% O_2 that had first passed through H_2SO_4 and NaOH solutions. The pH of solution that had not been exposed to tissue was about 7. Transepithelial PD was measured between two Ringer-agar bridges located within 3 mm of the tissue surface. Calomel cells connected the bridges to a high-impedance voltmeter. Current was passed from an external source via silver-silver chloride electrodes and Ringer-agar bridges to clamp the spontaneous transepithelial PD to zero (short-circuit current). The short-circuit current was corrected for solution resistance between the tips of PD-detecting bridges (automatic voltage clamps, UNC Dept. of Medical Electronics). PD and short-circuit current were recorded every 20 min. When tissues were continuously short-circuited, PD was recorded 45 s after the circuit was opened. Current was recorded 45 s after short-circuiting in open-circuit experiments. Because the PD across the bronchi was substantially lower than the tracheal PD, the resistive characteristics of the bronchi were also estimated from the current-voltage relationship that was measured at the end of each experiment. Like the trachea, the bronchi responded as an ohmic resistor over a PD range of ±50 mV. In most experiments, six preparations were run simultaneously. The order of tissue preparation and mounting was rotated to avoid preincubation bias. No tissues were discarded.

The pH of unbuffered solutions that bathed tracheal preparations was monitored by combined glass-reference electrodes connected to a high-impedance voltmeter. The electrodes were placed in the bathing solution reservoir of the gas-lifts. After 1 h the pH of each bathing solution was titrated with standardized NaOH or HCl to the initial value that was recorded after insertion of the electrode into the bath.

Isotope flux. ^{42}K (NC State University reactor, 15 μCi), ^{22}Na, and ^{36}Cl (New England Nuclear, 7.5 and 10 μCi, respectively) were added to either the luminal, i.e. mucosal (m), or submucosal (s) bathing solution about 30 min after mounting. Chemical carrier added with the isotope increased bathing solution ion concentrations by less than 1 meq/l. Because bioelectric properties were stable for 60–140 min after mounting (see RESULTS), isotope fluxes were measured during four 20-min intervals. In some experiments ouabain (Sigma) was added to the submucosal bath (final concentration = 3×10^{-5} M) 80 min after the addition of isotopes to the source bathing solution. Isotope flow was monitored for an additional 80 min. Data from the first 20-min collection period after ouabain were not included. Samples were taken from the source (0.05 ml) and sink (1.0 ml). The latter were replaced with an equal volume of "cold" Ringer solution. One milliliter of Ringer solution was added to all source samples to equalize the quench. All samples were counted immediately in a gamma spectrometer. All samples and standards were mixed with scintillation solution (Aquasol II, New England Nuclear) and counted in a liquid scintillation counter (Packard, Tri-Carb 3385). The uniformity of sample quench was monitored by the automatic external standard method. The ^{42}K counting rate was calculated by subtracting from the total counts measured immediately in the gamma spectrometer the ^{22}Na counts recorded 7 days after the experiment. Radiochloride activity was estimated by multiplying the ^{22}Na-counting rate in the gamma counter by the spill ratio for ^{22}Na-only standards and subtracting the product from the beta counting rate. Unidirectional fluxes and permeability coefficients were calculated from conventional relationships (13, 14).

Morphology. At the end of an experiment random tissues were fixed in the chamber with formaldehyde/paraformaldehyde. Specimens were embedded in Epon, 1-μm sections were cut and stained with toluidine blue, and photomicrographs were taken of stained sections (26).

Statistical analysis. Thirteen animals were used in the short-circuit studies. Because paired tissues from each region could not be obtained in four animals, the data were pooled and statistical analyses performed with *t* tests for means of independent samples. Data from the open-circuit experiments were treated similarly. Drug-induced changes in properties of the same tissue were assessed by paired *t* tests. The lines that describe the relationships between conductances and unidirectional permeability coefficients were fitted by the method of least squares (Figs. 3 and 4).

RESULTS

Figure 1 shows photomicrographs of tracheal and fourth- to sixth-generation bronchial tissues that are reproduced at identical magnification. Compared with epithelial cells of the trachea, bronchial epithelial cells are shorter but of similar cross-sectional diameter. Bron-

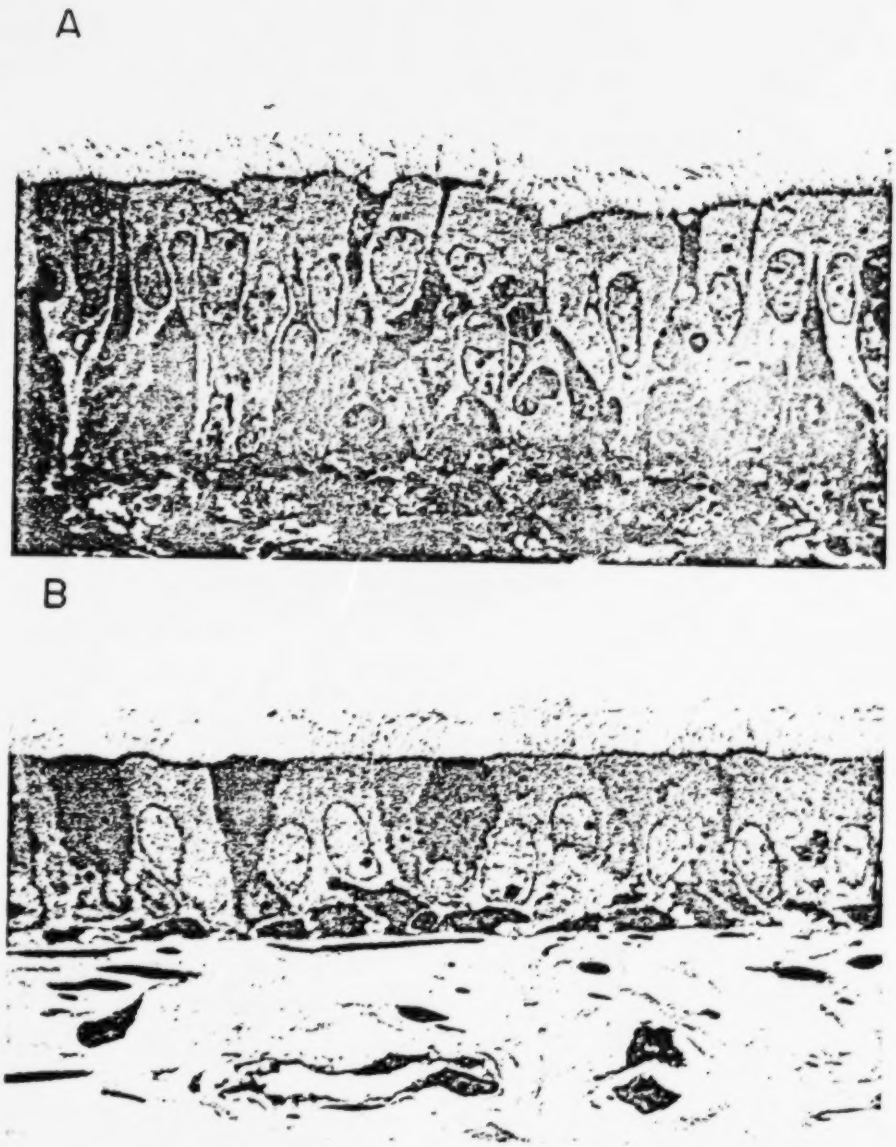

FIG. 1. Photomicrographs of thin sections of excised canine trachea (*A*) and subsegmental bronchus (*B*) stained with toluidine blue O (X1,345). Tissues fixed in Ussing chambers after incubation for 140 min.

chial goblet cells appear to be more prominent with numerous intracellular granules.

The 140-min course for the conductance and short-circuit current of the three airway regions under short-circuit conditions is shown in Fig. 2. Bioelectric properties changed no more than 15% during this period. Tissues under open circuit exhibited a comparable stability.

Table I presents the flux data and bioelectric properties for short-circuited airway regions. Isotope flows, like the bioelectric properties, were stable so that values for four sampling periods were averaged. The data for the trachea are similar to those reported by others who have used chambers with larger apertures. Compared with the spectrum of values in the literature, PD was relatively high and conductance low (1, 2, 9, 10, 22, 23). Net Cl^- secretion toward the lumen and a smaller flow of Na^+ in the absorptive direction were identified. The sum of the partial ionic conductances for the apparent passive flow of Na^+ and Cl^- ($J_{Na}^{s\rightarrow m} + J_{Cl}^{m\rightarrow s}$) approximated the measured electrical conductance and the total active translocation of Na^+ and Cl^- ($J_{Na}^{act.} + J_{Cl}^{act.}$) accounted for the measured short-circuit current. Unidirectional flows of K^+ were symmetric.

Whereas the short-circuit current in the main-stem bronchi approximated that in the trachea, the membrane conductance and apparent passive permeation of Na^+ ($J^{s\rightarrow m}$) and Cl^- ($J^{m\rightarrow s}$) were substantially greater and the PD lower. Significant Cl^- secretion was measured. The differences between the unidirectional Na^+ fluxes, although not significant, suggested active absorption. Again, the sum of $J_{Na}^{s\rightarrow m}$ and $J_{Cl}^{m\rightarrow s}$ was equivalent to conductance, the sum of $J_{Cl}^{act.}$ and apparent $J_{Na}^{act.}$ flows accounted for the short-circuit curent, and the net K^+ translocation was not detected.

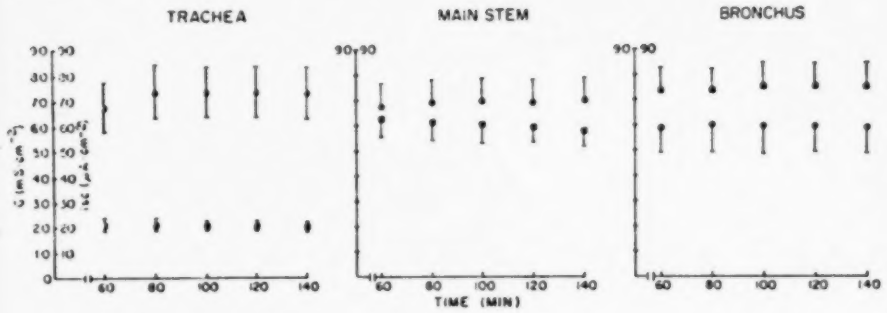

FIG. 2. Time course of bioelectric properties of short-circuited excised canine airways mounted in Ussing chambers. *Squares*, direct-current conductance (G); *circles*, short-circuit current (I_{sc}). *Each point* represents mean values for tissues excised from at least 8 dogs. *Vertical lines* depict SE.

TABLE 1. *Bioelectric properties and ion flow across canine airways (short circuit)*

Airway Region	n	Experiment	Bioelectric Properties			Flux, $\mu eq \cdot cm^{-2} \cdot h^{-1}$		
			PD, mV	I_{sc}, $\mu A \cdot cm^{-2}$	G, $mS \cdot cm^{-2}$	Na^+	Cl^-	K^+
Trachea	11	m → s	34.5 ±3.0	71.8 ±10.4	2.08 ±0.26	1.80 ±0.41	1.78 ±0.43	0.064 ±0.019
	11	s → m	35.5 ±3.2	72.7 ±10.2	2.05 ±0.19	0.65 ±0.10	3.53 ±0.48	0.049 ±0.08
		Net				1.15*	−1.75*	0.015
Main stem	10	m → s	10.6 ±1.5	63.6 ±8.3	6.41 ±0.72	2.75 ±0.47	3.26 ±0.49	0.041 ±0.007
	10	s → m	13.2 ±2.7	74.7 ±10.2	5.68 ±0.71	1.80 ±0.34	4.36 ±0.54	0.043 ±0.008
		Net				0.95	−1.10*	−0.002
Bronchus	11	m → s	10.9 ±2.0	74.3 ±13.9	6.82 ±0.70	3.79 ±0.43	5.66 ±0.78	0.050 ±0.008
	11	s → m	8.9 1.4	62.5 10.6	7.05 1.01	1.34 0.36	5.94 0.87	0.085 0.012
		Net				1.95*	−0.28	−0.035*

Values are means ± SE. Radioisotope flowed from mucosa to submucosa (m → s) or submucosa to mucosa (s → m). PD, potential difference; I_{sc}, short-circuit current; G, conductance. * Net flow significantly different from zero ($P < 0.05$).

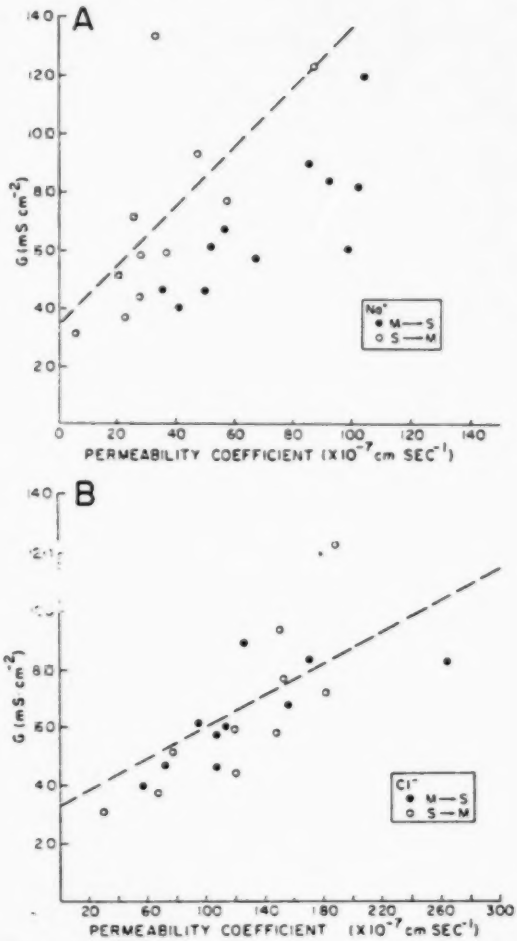

FIG. 3. Relationship between direct-current conductance (G) and unidirectional permeability coefficients for Na^+ (A) and Cl^- (B) flow across short-circuited excised canine subsegmental bronchus. m → s, flow from mucosal to submucosal bath; s → m, flow in opposite direction. *Each point* represents average value for 4 collection periods, 80–140 min after tisue was mounted in chamber. *Dashed lines* fitted to the s → m points in A and to the m → s points in B.

The smaller bronchi (~7-mm diam) exhibited a lower average PD (9.9 mV) than the main-stem bronchi. The lower PD appeared to result from a higher conductance, inasmuch as the short-circuit currents were similar. However, a different pattern of ion translocation was found. Net Na^+ movement in the absorptive direction (m → s) was the dominant ion flow, and net Cl^- secretion was not identified. The relationship of tissue conductance to unidirectional Na^+ and Cl^- movements, expressed as permeability coefficients, is shown in Fig. 3. Whereas all the points for $P_{Na^+}^{m \to s}$ fall to the right of line of best fit for the $P_{Na^+}^{s \to m}$ values (Fig. 3A), points for $P_{Cl^-}^{m \to s}$ and $P_{Cl^-}^{s \to m}$ cannot be separated into two populations (Fig. 3B). In contrast to the trachea and main-stem bronchi, K^+ secretion into the lumen was detected in the smaller bronchi. About 75% of the short-circuit current across segments of this region was accounted for by $J_{Na^+}^{net}$.

The data from open-circuit experiments are presented in Table 2. Bioelectric properties were similar to those from the short-circuit experiments, with the exception that the intermittently measured short-circuit current was 15–20% higher for each region. As previously reported by others (22, 23), net Cl secretion by the trachea was not detected. This unidirectional Cl flux symmetry in the presence of a transepithelial PD is compatible with active Cl^- transport. K^+ fluxes followed the expectation for passive movement in response to the electrochemical gradient. Compared with short-circuit conditions, submucosal-to-mucosal flow was raised and mucosal-to-submucosal flux was smaller. Net Na absorption was detected, despite a mean opposing PD of 38 mV. Fig. 4

TABLE 2. *Bioelectric properties and ion flow across canine airways (open circuit)*

Airway Region	n	Experiment	Bioelectric Properties			Flux, $\mu eq \cdot cm^{-2} \cdot h^{-1}$		
			PD, mV	I_{sc}, $\mu A \cdot cm^{-2}$	G, $mS \cdot cm^{-2}$	Na^+	Cl^-	K^+
Trachea	9	m → s	35.5	81.7	2.30	2.02	2.28	0.056
			±2.7	±12.4	±0.22	±0.37	±0.36	±0.006
	9	s → m	41.3	91.3	2.21	1.07	2.33	0.167
			±2.8	±14.9	±0.31	±0.17	±0.37	±0.027
		Net				95*	−0.05	−0.111*
Main stem	8	m → s	17.8	96.3	5.40	2.41	2.80	0.020
			±11.1	±17.2	±0.92	±0.48	±0.42	±0.004
	8	s → m	13.5	72.1	5.33	1.71	2.71	0.046
			±8.8	±10.2	±1.04	±0.27	±0.31	±0.003
		Net				0.70	0.09	0.026*
Bronchus	9	m → s	11.2	83.8	7.49	3.76	6.34	0.028
			±10.2	±73.9	±1.07	±0.26	±0.73	±0.002
	9	s → m	10.2	73.9	7.23	2.02	4.73	0.094
			±6.8	±6.8	±1.06	±0.40	±0.67	±0.006
		Net				1.74*	1.61	−0.066*

Values are means ± SE. Radioisotope flowed from mucosa to submucosa (m → s) or submucosa to mucosa (s → m). PD, potential difference; I_{sc}, short-circuit current; G, conductance. * Net flow significantly different than zero ($P < 0.05$).

depicts the relationship between conductance and the apparent unidirectional permeability coefficients for both Na^+ and Cl^-. Each $P_{Na^+}^{m \rightarrow s}$ falls to the right of a line of best fit for Na^+ flux in the passive direction, $P_{Na^+}^{s \rightarrow m}$ (Fig. 4*A*). The points for $P_{Cl^-}^{m \rightarrow s}$ and $P_{Cl^-}^{s \rightarrow m}$, however, do not appear to represent separate populations (Fig. 4*B*). In the mainstem bronchi a similar trend was observed, i.e., net K^+ flow was attributable to the electrical gradient and net Na^+ flow was in the absorptive direction. This Na^+ movement, however, was not statistically significant, nor was Cl^- secretion detected.

Because the 1.0 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ of Na^+ that was absorbed across the trachea under open-circuit conditions was not balanced by movement of other measured ions, we estimated the apparent net movement of protons or hydroxyl ions. Tracheas were bathed with an unbuffered Ringer that was initially free of bicarbonate and dissolved CO_2. The results are summarized in Table 3. In spite of a somewhat lower initial mucosal and higher submucosal bath pH than that of the conventional bathing solution, bioelectric properties were comparable. The pH of the mucosal bath fell and the submucosal pH rose progressively during the incubation period. Addition of proton equivalents to the mucosal bath averaged 0.06 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, about one-half the rate of hydroxyl equivalent addition to the submucosal solution.

In the small bronchi, net Na^+ reabsorption was clearly detectable. The absolute flow was somewhat less than in short-circuited tissues because $J_{Na^+}^{m \rightarrow s}$ was slightly smaller and $J_{Na^+}^{s \rightarrow m}$ slightly larger. Net K^+ flow toward the lumen was measured, and the ratio of K^+ fluxes exceeded that predicted from the PD (13 mV). In contrast, unidirectional Cl^- fluxes were not significantly different in these unpaired tissues although the trend in Cl^- movement appears to reflect the electrical gradient, i.e., $J_{Cl^-}^{m \rightarrow s}$ is greater than $J_{Cl^-}^{s \rightarrow m}$. Furthermore, $J_{Cl^-}^{net}$, although not significant, approximates $J_{Na^+}^{net}$.

The effects of ouabain on bioelectric properties and ion flow across trachea and bronchi under open-circuit conditions are summarized in Table 4. During the initial control period bioelectric properties and NaCl unidirectional fluxes were typical of the results reported in Tables 1 and 2. In particular, Na^+ absorption in the trachea was not balanced by a net flow of Cl^- in the same direction. However, Cl^- absorption across pairs of bronchial segments that were matched for conductance was significant in this series and exactly balanced the movement of Na^+. Ouabain effects on the tracheal bioelectric properties and Na^+ flow resemble the pattern reported by others (1, 32). PD and $J_{Na^+}^{m \rightarrow s}$ flux fell significantly, but conductance and $J_{Na^+}^{s \rightarrow m}$ in the passive direction (s → m) did not change. Cl^- fluxes were symmetric and increased significantly. The net result was inhibition of Na^+ reabsorption. Similar effects were noted in the bronchi except that conductance was increased, and both Na^+ and Cl^- absorption were abolished.

DISCUSSION

Our experiments were designed to compare and characterize ion permeability in accessible regions of canine airways above and below the area of minimal cross-

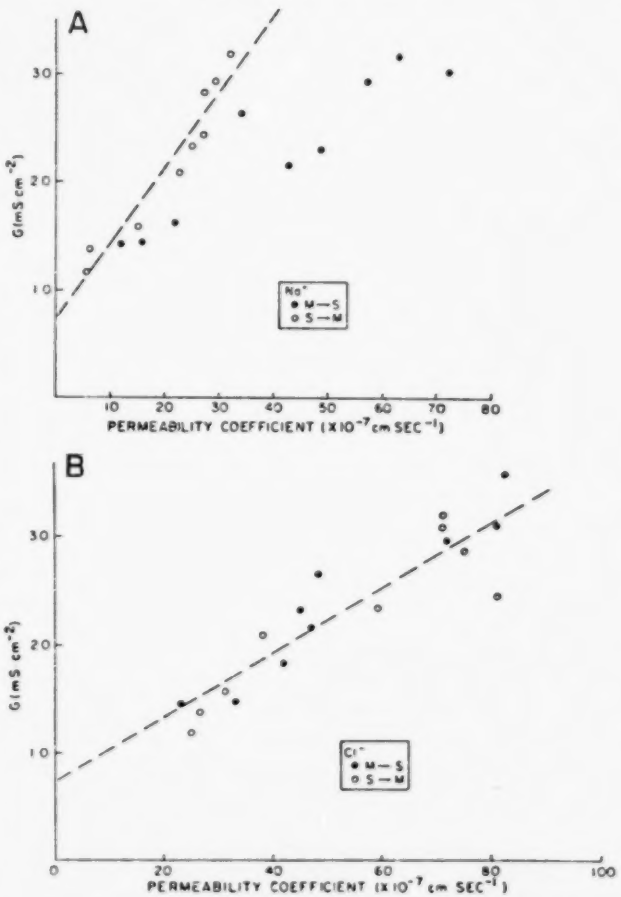

FIG. 4. Relationship between direct-current conductance (*G*) and apparent unidirectional permeability coefficients for Na^+ (*A*) and Cl^- (*B*) flow across excised canine trachea under open-circuit conditions. *Points* and *dashed line* are described in Fig. 3.

TABLE 3. *Bioelectric properties of and acid or base addition by the excised canine trachea*

Bioelectric properties	
Conductance, mS·cm^{-2}	2.98 ± 0.15
Short-circuit, current, μA·cm^{-2}	74 ± 8.8
Δ Solution pH, units·h^{-1}	
Mucosal (n = 11)	−0.11 ± 0.04
Submucosal (n = 4)	+0.33 ± 0.18
Rate of addition, μeq·cm^{-2}·h^{-1}	
Mucosal (acid*) (n = 11)	0.06 ± 0.01
Submucosal (base†) (n = 4)	0.12 ± 0.02

Values are means ± SE. Tissues were bathed by unbuffered solutions for at least 60 min. Initial mean mucosal pH, 6.85 ± 0.07; submucosal pH, 7.72 ± 0.18. * Estimated from the volume of standardized NaOH required to return bathing solution pH to the initial value; † Estimated from the volume of standardized HCl required to return bathing solution pH to the initial value.

TABLE 4. *Effects of ouabain on bioelectric properties and ion flow across excised canine airways (open circuit)*

Tissue	Parameter and Flux Direction	Before Ouabain	After Ouabain (submucosal, 3 × 10^{-5} M)
Trachea (n = 8)	PD, mV		
	m → s	32.0 ± 4.6	1.9 ± 0.4*
	s → m	36.4 ± 4.3	2.2 ± 0.7*
	Conductance, mS·cm^{-2}		
	m → s	2.28 ± 0.42	2.36 ± 0.88
	s → m	2.08 ± 0.36	1.86 ± 0.68
	Na^+ flux, μeq·cm^{-2}·h^{-1}		
	m → s	1.95 ± 0.35	1.12 ± 0.24*
	s → m	0.93 ± 0.32	0.93 ± 0.28
	Net	1.02 ± 0.24†	0.19 ± 0.23
	Cl^- flux, μeq·cm^{-2}·h^{-1}		
	m → s	2.57 ± 31	3.61 ± 0.33*
	s → m	2.28 ± 0.27	3.72 ± 0.45*
	Net	0.29 ± 0.31	−0.09 ± 0.29
Bronchi (n = 7)	PD, mV		
	m → s	7.3 ± 1.8	0.1 ± 0.1*
	s → m	9.9 ± 3.0	0.2 ± 0.1*
	Conductance, mS·cm^{-2}		
	m → s	8.00 ± 1.00	12.8 ± 1.3*‡
	s → m	7.61 ± 0.80	13.5 ± 1.4*‡
	Na^+ flux, μeq·cm^{-2}·h^{-1}		
	m → s	3.01 ± 0.26	1.70 ± 0.22*
	s → m	1.82 ± 0.46	1.67 ± 0.33
	Net	1.15 ± 0.29†	0.03 ± 0.25
	Cl^- flux, μeq·cm^{-2}·h^{-1}		
	m → s	5.56 ± 0.48	6.63 ± 0.60*
	s → m	4.37 ± 0.37	6.74 ± 0.41*
	Net	1.19 ± 0.35†	−0.09 ± 0.51

Values are means ± SE. Significantly different ($P < 0.05$, paired t test): * from value before ouabain addition; † from zero ($P < 0.05$, paired t test); ‡ from current-voltage plot 60 min after ouabain addition.

sectional area. Because the airways epithelia were studied in vitro, it is likely that the electrolyte transport and permeability determined in our study approximate "basal" function, i.e., a functional state uninfluenced by neural reflex arcs, blood-borne humoral agents, or blood flow.

Distinctly different regional bioelectric properties and ion permeabilities were noted (Table 1). In the trachea, the bioelectric properties were similar to those reported by others (1, 2, 22, 32). The relatively larger circumference-to-surface area ratio of the smaller aperture chambers (0.53 cm^2) used in this study was not associated with a higher membrane conductance than those reported for larger chambers (1.1–2.0 cm^2). In addition, PDs recorded with these chambers were similar to our in vivo measurements of tracheal PD (4). Consequently, it is unlikely that edge damage substantially affected our results. The concordance of in vivo and in vitro PD also argues against edge damage of the excised main-stem and subsegmental bronchi.

The well-documented pattern of Cl^- secretion toward the lumen of the trachea and a smaller flow of Na^+ toward the submucosa was found under short-circuit conditions (Table I). The main-stem bronchus was characterized by a substantially higher membrane conductance and passive ion flow, a lower PD, and a pattern of active Na^+ and Cl^- flow similar to that noted in the trachea. Asymmetries in K^+ unidirectional flux were not detected in either region.

Subsegmental bronchi were characterized by a relatively low PD and high conductance. The pattern of electrolyte flow in this region was different from that of the more central airways. Under short-circuit conditions $J^{net}_{Na^+}$ was nearly twice the Na^+ absorption in the trachea and accounted for a large fraction of the short-circuit current. Cl^- secretion was not detected, whereas K^+ was secreted. Finally, a portion of the short-circuit current was not accounted for by the total measured net ion flow. This residual short-circuit current may reflect small differences between large unidirectional flows. Hence, $J^{net}_{Na^+}$ may have been underestimated or a small $J^{net}_{Cl^-}$ obscured. Alternatively, the residual current may be accounted for by the net flow of an ion species that was not measured. Canine airway liquid pH has been reported to approximate 8 (25), and we have measured high HCO_3^- concentrations (50 meq/l) in canine bronchial-surface liquid (6). Accordingly, HCO_3^- may be secreted by the bronchial epithelium and contribute to the in vitro short-circuit current.

The open-circuit experiments were designed to evaluate ion flows in the presence of an electrical driving force that approximates the in vivo PD (4). Ion flow across canine trachea under open circuit has been described by several investigators (22, 23), so that we expected our results to confirm previous observations and serve as a basis for comparison with flows in other regions. The results in Tables 2 and 4 and in Fig. 4 confirm the reports that net Cl^- secretion by the trachea is reduced to zero, but Na^+ flow in the absorptive direction (m → s), although lower than the transport under short-circuited conditions, was easily detected. This aborptive flow was abolished by ouabain. Furthermore, our preliminary studies have revealed that tracheal Na^+ absorption under open circuit in vitro is widespread among species (e.g., rabbit, sheep, and monkey). Whether this discrepancy between our results and those of others reflects differences in levels of variance in the data or in experimental time course is not clear. The unidirectional flux of Na^+

from lumen to submucosa was numerically greater than flow in the opposite direction in two previous studies (22, 23). These reports, however, did not normalize open-circuit ion permeability to conductance, as we did in Fig. 4. Some of the studies by others focused on ion flow that was measured 6–8 h after tracheal tissue was mounted in the chamber. We have noted no obvious change in pattern of ion flow across tracheas during this period, but we have not studied the effects of the duration of in vitro incubation systematically.

Our results may provide insight into the link between Na^+ and Cl^- movements across the "resting" trachea. Several investigators have suggested that Cl^- secretion is dependent on Na^+ movement (1, 11, 28, 33). Coupled NaCl entry across the basal-lateral membrane of the epithelial cells has been proposed. This entry is driven by the Na^+ electrochemical gradient generated by active Na^+ extrusion across the same membrane region. Cl^- that enters with Na^+ exits passively across the luminal membrane down its electrochemical gradient. The persistence of $J^{net}_{Na^+}$ in open-circuit conditions implies that the Na^+ "pump" may have a larger electromotive force than the open-circuit potential. The reported electromotive force of most Na^+ pumps approximates 120 mV (7, 15, 34). Al-Bazzaz and Al-Awqati (1) have suggested that the slope change in the current-voltage plot for canine trachea at 120 mV may correspond to the Na^+ equilibrium potential. Hence, the persistence of Na transport across canine trachea with an open-circuit voltage of 30–40 mV is not surprising. The disappearance of net Cl^- secretion under open-circuit conditions implies that Cl^- flow follows the change in the electrochemical gradient across the cell membrane induced by the open-circuit PD. Precise estimates of the driving forces for Cl^- movement cannot be made because intracellular Cl activity has not been measured in canine tracheal epithelium. For the 10 mV (cell negative) lumenal membrane PD (12) to drive Cl^- into the lumen, the lumenal bathing solution to intracellular Cl^- activity ratio cannot exceed 1.5. Consequently, open-circuit conditions may result in an electrochemical driving force across the lumenal barrier that is precisely balanced by the tendency for the transmural voltage to drive anions through passive paths toward the submucosa.

Electroneutrality under open-circuit conditions requires that the net flow of Na^+ be matched by net anion movement toward the submucosa and/or cation flux in the opposite direction. Since Cl^- unidirectional flows were equal, other sources of net charge flow must be considered. The K^+ flux asymmetry accounted for about 0.1 of the missing 0.9 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, and proton addition to the lumenal solution added no more than 0.1 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. Apparent OH^- addition to (or proton consumption in) the submucosal bath contributed another 0.2 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. However, cells and/or materials in the submucosal interstitium may have been responsible for the latter changes. The unidentified ion that is most likely to balance Na^+ transport is bicarbonate. Because bicarbonate is in equilibrium with dissolved CO_2 in bicarbonate-buffered solutions, the movement of bicarbonate ion is difficult to assess. When a permeability coefficient equal to that of Cl^- is assigned to bicarbonate, a passive bicarbonate flux asymmetry of 3 to 1 and a net flow of 0.4 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ is predicted from the electrical PD. In preliminary experiments with 5 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer or unbuffered solutions, net Cl^- flow came closer to balancing Na^+ absorption under open-circuit conditions when bicarbonate was absent (unpublished observations).

In smaller bronchi, Na^+ absorption was measured under open-circuit conditions. Persistence of net Na^+ transport was expected because the change in PD between open-circuit and short-circuit conditions in this region is small. Net K^+ flow into the lumen increased slightly, and the flux ratio exceeded the prediction for passive flow. Cl^- appears to move passively, and the net flow balanced Na^+ transport. As noted above, bicarbonate may be secreted in this region, but we have no direct evidence for this contention. In addition, the high bicarbonate concentration that has been measured in liquid sampled from bronchial surfaces in vivo could have originated from more distal sites. It is clear that NaCl absorption dominates electrolyte transport across the bronchi under open-circuit, a condition that closely resembles the in vivo electrical gradient.

The relationship of mucosal morphology to differences in ion transport and permeability in trachea and bronchial regions is difficult to evaluate. For example, segmental airways in dogs have been reported to contain fewer glands than the trachea (17). The relevance of this difference in gland density to the pattern of ion transport reported herein is unclear because the division of transport "labor" between surface epithelial and glandular cells in a single airway region, the trachea, has not been defined. For example, the model of Na^+ absorption and Cl^- secretion proposed for the tracheal epithelium attempts to explain ion flows by processes that reside in the same cell. Further Widdicombe et al. (31) reported that the magnitude of ouabain binding to surface and glandular epithelial cells was similar, implying similar ATPase levels and, perhaps, relatively uniform Na^+ transport.

However, patterns of response to drugs imply the participation of cells from separate sites in the epithelium. For example, acetylcholine selectively increases Cl^- secretion and reverses the direction of net Na^+ flow across canine trachea resulting in an electrically silent secretion (22). Because cholinomimetic agents induce glandular secretion in vivo (8), it is tempting to assign this pattern of in vitro ion translocation to glands. A smaller cholinergic stimulation would be expected in regions of lower gland density (bronchi). Preliminary results support this prediction (5).

A different pattern of ion flux is induced by β-adrenergic agonists or methyl xanthines in canine trachea. These agents raise Cl^- secretion and the response is electrogenic, i.e., PD and short-circuit current increase (2). The PD response can be detected in vivo by bridges that make contact with and apply drugs directly to the surface of the epithelium (4). These results suggest that secretion induced by β-adrenergic agents may emanate from surface epithelial cells. However, β-agents do not induce a change Cl^- secretion or raise short-circuit current of

excised canine bronchi in vitro (unpublished observations). Accordingly, it is clear that regional differences in transport must reflect differences in the density and nature of the functional units, but the contribution of each component remains to be defined.

Similarly, the morphologic correlates of the higher membrane conductance in bronchial regions are not clear. In particular, the incidence of structures that are thought to be associated with paracellular routes of passive flow has not been quantified. Our observation (Fig. 1) that the average cell diameter is similar in trachea and bronchi makes it unlikely that the regional differences in conductance were simply a function of differences in the total surface area occupied by cell-to-cell junctions (10).

Our results help define basal ion permeability and transport across the epithelium of the large airways, but the balance of salt and water across each of these regional barriers is clearly more complicated in vivo. The surfaces of the large bronchi are thought to receive liquid that is secreted by the small airways and/or alveolar surfaces and that is swept toward the mouth by ciliomotion. Neither the electrolyte composition or secretion rate of this liquid nor its cephalad flow rate have been precisely defined. The bronchi have the capacity to modify the composition of the surface liquid by reabsorbing sodium chloride and, presumably, water. K^+ and perhaps bicarbonate are added. Preliminary studies indicate that cholinergic and adrenergic neurohumors slow but do not reverse salt reabsorption in this region (5, and unpublished observations).

Surface fluid then moves on toward the trachea where basal Na^+ reabsorption would be expected to drive water into the interstitium. This process, is opposed in vivo by humidification of inspired air, which increases the osmolality of surface liquid (6, 21), and by fluctuations in the local concentrations of neurohumors and autocoids (e.g., histamine), which stimulate secretion of liquid from glands and, perhaps, surface epithelial cells (22).

Our study supports the predictions of Kilburn (3, 19) that reabsorption dominates the pattern of ion flow in the large bronchi, the region of minimal cross-sectional surface area of "choke point." Surface liquid composition and volume in the trachea, however, appear to reflect a complex balance of active and passive flows. Basal absorption of salt throughout the upper airways suggests that this flow plays an important role in the regulation of the depth of the sol layer. Accordingly, abnormalities in mucociliary clearance and excessive airway secretions may involve dysfunction of airway liquid absorption as well as secretion.

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